Application No. 10/031,331

Applicants: Akioy YAMADA et al.

The listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) A method for screening DNA encoding proteins having the activity of

improving environmental stress tolerance wherein candidate cDNA derived from cDNA library

is introduced into host cells, the obtained transformed cells are cultured under the conditions

where the host cells cannot substantially grow, the clones grown after culturing are selected, and

the candidate cDNA introduced from the selected clones is isolated.

2. (Original) A method for screening DNA encoding proteins having the activity of

improving environmental stress tolerance wherein candidate cDNA derived from cDNA library

is introduced into host cells, the obtained transformed cells are cultured under conditions where

the host cells cannot substantially grow, the clones grown after the culturing are selected, the

candidate cDNA introduced from the selected clones is isolated, the isolated candidate cDNA is

introduced into the isolated cDNA, the mutant cDNA is introduced into host cells, and the

process of selecting is repeated one or more under stringent conditions of selecting mutant cDNA

than the selecting condition.

3. (Previously Presented) The method for screening according to claim 1, wherein the

environmental stress is one or more of chemical substance stress, high temperature stress, low

temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress,

or osmotic pressure stress.

4. (Original) The method for screening according to claim 3, wherein the chemical

substance stress is salt stress.

-2-

Application No. 10/031,331

Applicants: Akioy YAMADA et al.

5. (Previously Presented) The method for screening according to claim 1, wherein the

host cell is a coliform.

6. (Original) The method for screening according to claim 5, wherein the coliform is

SOLR strain.

7. (Previously Presented) The method for screening according to claim 1, wherein an

environmental condition where host cells cannot substantially grow is 350mM or more of salt

concentration.

8. (Previously Presented) DNA encoding proteins having the activity of improving

environmental stress tolerance wherein the DNA is obtained according to claim 1.

9. (Original) DNA encoding proteins having the activity of improving environmental

stress tolerance according to claim 8, wherein the environmental stress is one or more of stresses

selected from chemical substance stress, high-temperature stress, low-temperature stress,

freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress, or osmotic

pressure stress.

10. (Original) DNA encoding proteins having the activity of improving the

environmental stress tolerance according to claim 9, wherein the chemical substance stress is salt

stress.

-3-

11. (Previously presented) DNA encoding proteins having the activity of improving the

environmental stress tolerance according to claim 8, wherein the proteins having the activity of

improving the environmental stress tolerance are derived from plants.

12. (Original) DNA encoding proteins having the activity of improving the

environmental stress tolerance according to claim 11, wherein the plant is Bruguiera sexangla,

Avicennia marina, Sueada japonica, Salsola komarovii, or Mesembryanthemum crystallinum.

13. (Original) DNA encoding proteins according to any one of the following (a) to (c):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 2,

(b) a protein comprising a sequence of amino acids having 70% or more of

homology with the sequence of amino acids shown in Seq. ID No. 2, and having the activity of

tolerance at least against salt stress,

(c) a protein having a sequence of amino acids wherein one or more of amino acids

are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 2, and

having the activity of improving tolerance at least against salt stress.

14. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 1, or its complementary sequence.

15. (Original) DNA hybridized with the DNA according to claim 14 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

-4-

- 16. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 4,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 4, and having the activity of improving tolerance at least against salt stress.
- 17. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 3 or its complementary sequence.
- 18. (Original) DNA hybridized with the DNA according to claim 17 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
 - 19. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 6,
- (b) a protein comprising the sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 6, and having the activity of improving tolerance at least against salt stress.
- 20. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 5 or its complementary sequence.

- 21. (Original) DNA hybridized with the DNA according to claim 20 under stringent conditions, and encoding proteins comprising the activity of improving tolerance at least against salt stress.
 - 22. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 8,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 8, and having the activity of improving tolerance at least against salt stress.
- 23. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 7 or its complementary sequence.
- 24. (Original) DNA hybridized with the DNA according to Claim 23 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
 - 25. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising a sequence of amino acids shown in Seq. ID No. 10,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 10, and having the activity of improving tolerance at least against salt stress.

26. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 9 or its complementary sequence.

27. (Original) DNA hybridized with the DNA according to claim 26 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

28. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein having the sequence of amino acids shown in Seq. ID No. 12,

(b) a protein having a sequence of amino acids wherein one or more of amino acids

are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 12, and

having the activity of improving tolerance at least against salt stress.

29. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 11 or its complementary sequence.

30. (Original) DNA hybridized with the DNA according to claim 29 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

31. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein having the sequence of amino acids shown in Seq. ID No. 14,

(b) a protein having a sequence of amino acids wherein one or more of amino acids

are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 14, and

having the activity of improving tolerance at least against salt stress.

-7-

Applicants:

32. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 13 or its complementary sequence.

33. (Original) DNA hybridized with the DNA according to claim 32 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

34. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 16,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 16,

and having the activity of improving tolerance at least against salt stress.

35. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 15 or its complementary sequence.

36. (Original) DNA hybridized with the DNA according to claim 35 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

37. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 18,

-8-

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 18,

and having the activity of improving tolerance at least against salt stress.

38. (Original) DNA having part or whole of the sequence of bases shown in Seq. ID No.

17 or its complementary sequence.

39. (Original) DNA hybridized with the DNA according to Claim 38 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

40. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 20,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 20,

and having the activity of improving tolerance at least against salt stress.

41. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 19 or its complementary sequence.

42. (Original) DNA hybridized with the DNA according to claim 41 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

-9-

- 43. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 22,
- (b) a proteins comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and having the activity of improving tolerance at least against salt stress.
- 44. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 21 or its complementary sequence.
- 45. (Original) DNA hybridized with the DNA under stringent conditions according to claim 44, and encoding proteins having the activity of improving tolerance at least against salt stress.
 - 46. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 24,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having activity of improving tolerance at least against salt stress.
- 47. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 23 or its complementary sequence.

- 48. (Original) DNA hybridized with the DNA according to Claim 47 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
 - 49. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 26,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress.
- 50. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 25 or its complementary sequence.
- 51. (Original) DNA hybridized with the DNA according to Claim 50 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
 - 52. (Original) DNA encoding proteins according to any one of the following (a) or (b):
 - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 28,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 28, and having the activity of improving tolerance at least against salt stress.

53. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 27 or its complementary sequence.

54. (Original) DNA hybridized with the DNA according to claim 53 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

55. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 30,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in. ID No. 30, and

having the activity of improving tolerance at least against salt stress.

56. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 29 or its complementary sequence.

57. (Original) DNA hybridized with the DNA according to Claim 56 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

58. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 32,

-12-

Application No. 10/031,331

Applicants: Akioy YAMADA et al.

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 32,

and having the activity of improving tolerance at least against salt stress.

59. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 31 or its complementary sequence.

60. (Original) DNA hybridized with the DNA according to claim 59 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

61. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 34,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 34,

and having the activity of improving tolerance at least against salt stress.

62. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 33 or its complementary sequence.

63. (Original) DNA hybridized with the DNA according to Claim 62 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

-13-

- 64. (Currently Amended) \underline{A} DNA encoding \underline{a} proteins according to any one of the following (a) or (b):
- (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, and having the activity of improving tolerance at least against salt stress.
- 65. (Currently Amended) A DNA comprising part or whole all of the sequence of bases shown in Seq. ID No. 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, or 63, or its complementary sequence.
- 66. (Currently Amended) A DNA hybridized which hybridizes with the DNA according to claim 65 under stringent conditions, and encoding encodes proteins having the activity of improving tolerance at least against salt stress.
- 67. (Currently Amended) A method for improving environmental stress tolerance, wherein the DNA according to elaim 8 any one of claims 64-66 is used.
- 68. (Original) The method for improving the environmental stress tolerance according to claim 67, wherein the environmental stress is one or more of chemical substance stress, high temperature stress, low temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress, and/or osmotic pressure stress.

69. (Original) The method for improving environmental stress tolerance according to

claim 68, wherein the chemical substance stress is salt stress.

70. (Original) A protein comprising of the sequence of amino acids shown in Seq. ID

No. 2.

71. (Original) A protein having 70% or more of homology with the sequence of amino

acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt

stress.

72. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 2, and having the activity of improving tolerance at least against salt stress.

73. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

4.

74. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 4, and having the activity of improving tolerance at least against salt stress.

75. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

6.

-15-

76. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 6, and having the activity of improving tolerance at least against salt stress.

77. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

8.

78. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 8, and having the activity of improving tolerance at least against salt stress.

79. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

10.

80. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 10, and having the activity of improving tolerance at least against salt stress.

81. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

12.

82. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 12, and having the activity of improving tolerance at least against salt stress.

-16-

- 83. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 14.
- 84. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 14, and having the activity of improving tolerance at least against salt stress.
- 85. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 16.
- 86. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 16, and having the activity of improving tolerance at least against salt stress.
- 87. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 18.
- 88. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 18, and having the activity of improving tolerance at least against salt stress.
- 89. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 20.

- 90. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 20, and having the activity of improving tolerance at least against salt stress.
- 91. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 22.
- 92. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and having the activity of improving tolerance at least against salt stress.
- 93. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 24.
- 94. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having the activity of improving tolerance at least against salt stress.
- 95. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 26.

- 96. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress.
- 97. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 28.
- 98. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 28, and having the activity of improving tolerance at least against salt stress.
- 99. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 30.
- 100. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 30, and having the activity of improving tolerance at least against salt stress.
- 101. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 32.
- 102. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 32, and having the activity of improving tolerance at least against salt stress.

103. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

34.

104. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 34, and having the activity of improving tolerance at least against salt stress.

105. (Currently Amended) A protein comprising the sequence of amino acids shown in

Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64.

106. (Currently Amended) A protein comprising a sequence of amino acids wherein one

or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in

Seq. ID No. 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, and having the activity of

improving tolerance at least against salt stress.

107. (Previously Presented) An antibody specifically bound to the protein according to

claim 70.

108. (Previously Presented) An antibody specifically bound to the protein according to

claim 73.

109. (Previously Presented) An antibody specifically bound to the protein according to

claim 105.

-20-

- 110. (Previously Presented) The antibody according to claim 107, wherein the antibody is a monoclonal antibody.
- 111. (Previously Presented) A vector comprising the DNA encoding proteins having the activity of improving tolerance against environmental stresses according to claim 8.
 - 112. (Previously Presented) A vector comprising the DNA according to claim 13.
 - 113. (Previously Presented) A vector comprising the DNA according to claim 16.
- 114. (Currently Amended) A vector comprising the DNA according to elaim any one of claims 64-66.
- 115. (Currently Amended) A transformed cell obtained by introducing the vector according to claim 111 114 to a host cell.
- 116. (Original) A transformed cell according to claim 115, wherein the host cell is a plant cell.
- 117. (Currently Amended) A method for producing proteins a protein having the activity of improving environmental stress tolerance, wherein the transformed cells according to claim 115 is cultured, and a recombinant proteins—are—is collected from the transformed cells or the supernatant of the cultured liquid.

118. (Previously Presented) A transgenic plant obtained by introducing the DNA

according to claim 8 encoding proteins having the activity of improving environmental stress

tolerance, and by dividing, proliferating and redifferentiating the plant cell.

119. (Previously Presented) A transgenic plant obtained by introducing the DNA

according to claim 13 encoding proteins having the activity of improving environmental stress

tolerance, and by dividing, proliferating and redifferentiating the plant cell.

120. (Previously Presented) A transgenic plant obtained by introducing the DNA

according to claim 16 encoding proteins having the activity of improving environmental stress

tolerance, and by dividing, proliferating and redifferentiating the plant cell.

121. (Currently Amended) A transgenic plant obtained by introducing the DNA

according to elaim any one of claims 64 to 66 to a plant cell and encoding proteins having the

activity of improving environmental stress tolerance, and by dividing, proliferating and

redifferentiating the plant cell.

122. (Currently Amended) A transgenic plant obtained by introducing the vector

according to claim 111 114, and by dividing, proliferating and redifferentiating the plant cell.

123. (Currently Amended) The transgenic plant according to claim 118121, wherein the

environmental stress is one or more of chemical substance stress, high temperature stress, low

temperature stress, frenzying stress, drought stress, ozone stress, ultraviolet stress, radiation

stress, and/or osmotic pressure stress.

-22-

- 124. (Original) The transgenic plant according to claim 123, wherein the chemical substance stress is salt stress.
- 125. (Currently Amended) A material for breeding derived from the transgenic plant according to claim 118121.